

616.9362
T.H.
C539a

PASA RA(HA) 1-70 RES.
HEW/PHS/CDC
PN-AAE-363

A COMPARATIVE EVALUATION OF
SULFALENE-TRIMETHOPRIM AND
SULPHORMETHOXINE-PYRIMETHAMINE
AGAINST FALCIPARUM MALARIA IN THAILAND

WILLIAM CHIN, DAVID M. BEAR, EDWARD J. COLWELL,
AND SANONG KOSAKAL

Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol 22, No. 3, May 1973
p 308-312

Printed in United States of America

Copyright © 1973 by The American Society of Tropical Medicine and Hygiene

A. I. U.
Reference Center
Room 1656 NS

A COMPARATIVE EVALUATION OF
SULFALENE-TRIMETHOPRIM AND
SULPHORMETHOXINE-PYRIMETHAMINE
AGAINST FALCIPARUM MALARIA IN THAILAND*

WILLIAM CHIN,† DAVID M. BEAR,‡ EDWARD J. COLWELL,§
AND SANONG KOSAKAL||

*The Thailand Malaria Operational Research Unit, Bangkok, Thailand, the Malaria Program,
Center for Disease Control, Health Services and Mental Health Administration,
U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333,
the U.S. Army Medical Component, SEATO, APO San Francisco 96346,
and Trad Provincial Hospital, Thailand*

Abstract. The efficacies of combinations of sulfalene-trimethoprim (SF-T) and sulphormethoxine-pyrimethamine (S-P) against falciparum malaria in Thailand were assessed. Thirty-one patients were given a single dose of SF-T (1 g SF and 0.5 g T) and 34 patients were given S-P (1 g S and 0.05 g P). Radical cures were observed in 84% of the patients given SF-T and in 91% of those given S-P. Clearances of fever and asexual parasitemias were similar. Factors of previous malaria infections as indicated by both the patients' medical history and by the presence of malaria fluorescent antibodies and prior antimalarial intake showed no apparent influence on treatment outcome. No significant toxic side reactions were observed in subjects administered either one of the combination treatments. A plan for the field use of the long-acting sulfonamide combinations is proposed.

Chloroquine-resistant falciparum malaria was first reported from Thailand in 1962.¹ Since that time, investigations performed in Thailand by Dr. Tranakchit Harinasuta,² SEATO Medical Research Laboratory,³ and Thailand Malaria Operational Research Unit⁴ have shown rates ranging from 50% to 100%.

Because of this problem of chloroquine resistance and in view of the fact that administration of quinine to large numbers of patients in the field is operationally impractical, the need in Thailand for a simple and effective regimen against falciparum malaria is most urgent. Presently, two sulfonamide combinations have the potential for meeting this need. These are: 1) sulfalene-trimethoprim (SF-T), and 2) sulphor-

methoxine (Fanasil,[®] Roche)-pyrimethamine (S-P).

The purpose of this study was to assess these two combinations against falciparum malaria in adult Thai males, with respect to rapidity of action, cure rate, the effect of preexisting malaria antibodies on cure rate, and possible side effects, especially side effects in individuals whose erythrocytes are deficient in glucose-6-phosphate dehydrogenase (G-6-PD) activity.

MATERIALS AND METHODS

The site selected for study was Trad Province, located in Southeast Thailand approximately 400 km from Bangkok (see Fig. 1). Previous investigations have shown that the majority of *Plasmodium falciparum* strains from this area are chloroquine resistant. In 1968, investigations of the chloroquine sensitivity of *P. falciparum* were conducted in an area 50 km north of Trad Hospital.⁵ Treatment failures following administration of chloroquine were observed in 8 (80%) of 10 subjects who received a total dosage of 25 mg (base)/kg and in 19 (100%) of 19 additional subjects who received a single dose at 10 mg (base)/kg. Just prior to the present study, Colwell and co-workers reported, from the same hospital, a chloroquine-resistance rate of 93% in

Accepted 28 September 1972

* This study was supported by the Agency for International Development, U.S. Department of State, and the U.S. Army, Department of Defense. This is contribution No. 1047 from the Army Malaria Research Program.

† Formerly Chief, TMORU. Present address: American Embassy (Central America Malaria Research Station), APO New York 09389.

‡ Formerly Epidemiologist, TMORU.

§ U.S. Army Medical Component, SEATO

|| Trad Provincial Hospital, Thailand.

41 infections examined by an in-vitro method,⁶ and a treatment failure rate of 58% in 36 subjects administered a combination consisting of chloroquine 1.5 g (base) plus quinine 540 mg 3 times daily for 3 days.⁷

Subjects for this evaluation were chosen from ill individuals seeking treatment at the Trad Provincial Hospital. Selected were adult males, 15 years of age or older, with a positive blood smear for falciparum malaria who, by history and physical examination, were found to be free of obvious evidence of renal, cerebral, or hepatic complications.

Initial laboratory determinations consisted of hematocrit and leukocyte count. A venous blood specimen was obtained for the assessment of possible erythrocytic G-6-PD deficiency by the Hyland G-6-PD fluorescent spot test and for assay of malaria antibody by the indirect fluorescent antibody (IFA) method. Subjects were then alternately assigned to one of the following two orally administered treatment regimens:

SF-T: Sulfalene 1 g and trimethoprim 0.5 g.

S-P: Sulphormethoxine 1 g and pyrimethamine 0.050 g.

Following treatment in the outpatient clinic, subjects were admitted to the medical ward, where they were observed initially for vomiting and, when required, given supportive care. Patients were discharged after at least 3 consecutive days of negative blood smear examinations. Following discharge, there were no significant differences between these treatment groups which might lead to differences in reexposure to malaria infections.

Hematocrits, leukocyte counts, and thick-thin blood smears were taken at least daily during the patients' hospitalization and weekly thereafter on days 7, 14, 21 and 28 following commencement of therapy. (The day of treatment was designated day 0.) The smears were stained with Giemsa. Parasites per mm³ were calculated by multiplying the number of parasites per 100 leukocytes by the factor obtained from each patient's leukocyte count taken on the same day. (Factor = leukocytes/mm³ of blood ÷ 100.) If 5 minutes of examination failed to disclose asexual parasites in the thick film, a smear was considered negative.

In this study, a radical cure was defined as the clearance of patent asexual parasitemia by day 7 with no recrudescence during the weekly follow-up

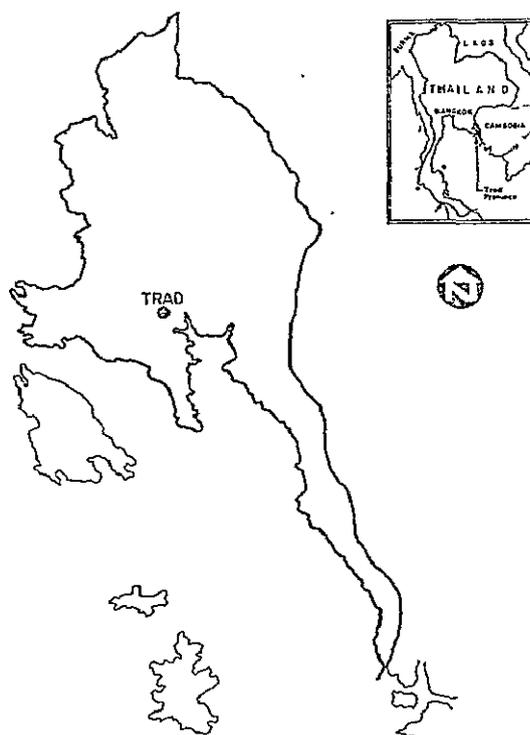


FIGURE 1. Map of Southeast Thailand showing the study site, Trad Province.

examinations to day 28. Failure was the lack of clearance of patent parasitemia by day 7 or recrudescence of patent infection during any of the weekly follow-up examinations after initial clearance.

RESULTS

Eighty-eight patients were selected for inclusion in the study. Adequate history from 75 of the patients indicated that 73% took some anti-malarials prior to their hospital admission. The medication most frequently utilized was chloroquine, although occasionally intramuscular quinine had been administered.

More than 90% of the subjects had been ill for an average duration of 6 days prior to hospital admission. Fewer than 10% stated that they had come to the hospital on the 1st day of illness or that they had been ill for greater than 1 month. Approximately 60% of the patients had a history of one or more previous malaria infections.

The pre-treatment observations of the two groups are summarized in Table 1. As indicated, the two groups were quite similar with respect

TABLE 1
Pre-treatment observations of study subjects

Treatment	No. cases	Age in years Range (average)	Weight in kg Range (average)	Asexual parasite count/mm ³ Range (geometric mean)
SF-T	45	15-50 (26.1)	25-90 (50.9)	59-120,054 (6,473)
S-P	43	15-51 (25.5)	31-63 (48.6)	271-125,400 (7,010)

to age, weight and initial parasite density. The results of the immediate response to treatment are summarized in Table 2. As noted, clearances of fever and asexual parasitemias were very similar. There was one case from each treatment group in which patent parasitemia was not cleared within 7 days.

Follow-up examinations for 1 month were accomplished in 65 of the 88 cases and the results are summarized in Table 3. Although the S-P regimen produced a higher cure rate than that of SF-T, the difference was not significant (Fisher's exact test, $P = 0.4$). In the 6 cases of treatment failure with delayed recrudescence, patent parasitemia was cleared initially within 2 to 4 days in the SF-T treated individuals, and within 6 to 7 days in the two patients given S-P.

Indirect fluorescent antibody tests were performed on sera from all individuals at the beginning of the study. Fifty-six (64%) of 88 subjects exhibited positive reactions in the IFA test, employing *P. falciparum* antigen. Among the 65 patients with 28 days follow-up, the treatment responses did not differ with the presence or absence of malaria antibody (Table 4).

The leukocyte count and hematocrit values of all subjects were within the usual range found in patients with malaria. G-6-PD deficiency was found in 11 (12.5%) of the 88 subjects. Comparison of the post-treatment hematocrit values of these 11 individuals with the other subjects disclosed no detectable difference.

TABLE 2
Immediate response to treatment

Treatment*	No. cases	Clearance of parasitemia (by day 7)		Average no. days to clear parasitemia	Average no. days to clear fever (100° F)
		No.	(%)		
SF-T	45	44	(97.8)	2.2	2.4
S-P	43	42	(97.7)	2.6	2.6

* Single dose administration of SF-T* (sulfalene, 1 g-trimethoprim, 0.5 g); S-P. (sulphormethoxine, 1 g-pyrimethamine, 0.05 g).

The effect of treatment on gametocytemia in 63 of the cases observed for 28 days is depicted in Table 5. Gametocytes appeared in both treatment groups approximately 6 days after treatment. In most of the cases, gametocyte densities were less than 1,000/mm³. A maximal density of 18,778/mm³ was observed in one exceptional case, 7 days after treatment with S-P.

Subjects in both groups tolerated the medications well, and no adverse side reactions were observed.

DISCUSSION

Interest in the use of sulfonamides as anti-malarials has been renewed recently. It derives from the finding that the potentiation of sulfonamides by the addition of a dihydrofolate reductase inhibitor results in effective treatment of falciparum malaria,⁸ including chloroquine resistant strains,⁹ at dosages which are both safe and easy to administer.

Of the two combinations evaluated in this study, sulphormethoxine-pyrimethamine has been used more extensively and for a longer period of time than the sulfalene-trimethoprim regimen. Using a single dose of sulphormethoxine (1 g) and pyrimethamine (50 mg), Chin et al.⁹ reported cures in all six volunteers infected with a multi-resistant strain of *P. falciparum* which originated from Thailand. In 1967, Harinasuta et al.,² using the same dosage, reported radical cures in 17 of 19 Thai patients infected with chloroquine-resistant falciparum malaria. Prior to the current study, the use of sulfalene-trimethoprim against

TABLE 3
Treatment results in patients followed for 1 month

Treatment	No. cases*	No. cured (%)	Day of recrudescence
SF-T	31	26 (83.9)	14, 28, 21, 28
S-P	34	31 (91.2)	28, 28

* Also includes the 2 immediate treatment failures noted in Table 2.

TABLE 4

Results of indirect fluorescent antibody (IFA) tests on pre-treatment sera against falciparum malaria in subjects with treatment success or failure

	Malaria antibody results		
	No. examined	No. positive ($\geq 1:20$)	% positive
Treatment success ^a	57	38	66.7
Treatment failure	8	4	50.0

^a At least 28 days of follow-up examination.

a Thai strain of falciparum malaria had been limited to the successful treatment by Clyde of a single volunteer infected with the Man strain.¹⁰

The results of our investigation showed that there were no significant differences between the responses of the falciparum strains to the two combinations. It is conceivable that previous experience with malaria and prior antimalarial treatment may have contributed to the favorable cure rates, but this was not supported by the data. The "immune" status of the subjects as determined by historical evidence of previous malaria infections and by the IFA method was similar (60% vs. 63.6%). The difference in the IFA positivity in those who were cured and in the cases judged as treatment failures (66.6% vs. 50%) was not statistically significant. The data therefore suggest that mere presence of malaria fluorescent antibody and/or history of previous experience with malaria had no demonstrable effect in the final determination of treatment success or failure. Likewise, there was no positive correlation between prior antimalarial intake and final outcome since all eight treatment failure cases admitted to antimalarial intake prior to hospital admission.

Since patients returned to malarious areas after only 7 days or less of hospitalization, the question of whether some of the delayed failures may actually represent reinfections is open to speculation. To further compound the difficulties of accurate assessment of results, it should be recognized that the use of the 30-day post-treatment observation period is too short. Chin et al.⁹ reported that in half of the delayed treatment failures in volunteers infected with falciparum malaria and given sulfonamides alone or with pyrimethamine, recrudescence of patent infection occurred between days 41 and 60.

The question of potential for development of resistance to this combination following wide-scale use, in light of present knowledge and experience, remains a perplexing one. Richards,¹¹ in his attempt to induce resistance of *P. berghei* to sulfonamides and pyrimidine combinations, concluded that the pyrimethamine-sulphormethoxine combination did not produce resistance as rapidly or to the same degree in comparison to each drug given alone or to a less active combination of trimethoprim-sulfalene.

Conversely, Verdrager et al.¹² reported that probable cross resistance to sulphormethoxine-pyrimethamine was observed in falciparum infections when the combination was given subsequent to treatment failure with Dapsone. Chin et al.¹³ confirmed this latter finding when they reported that following exposure to a single, non-curative dose of a mixture of cycloguanil pamoate and 4,4'-diacetyl-aminodiphenyl sulfone, only 1 out of 5 chloroquine resistant falciparum infections was cured with sulphormethoxine-pyrimethamine compared to a 100% cure rate in 6 cases infected with the same strains prior to exposure of the parasite to the cycloguanil pamoate-sulfone mixture.

TABLE 5

The effect of SF-T and S-P treatment on gametocytemia in cases followed for 1 month

Treatment	Total cases	Gametocytes present on initial smear		Gametocytes not observed during 28 days		Gametocytes developing after treatment	
		No.	(%)	No.	(%)	No.	(%)
SF-T	30	3	(10.0)	7	(23.3)	20	(66.6)
						range = 1-14 days average = 5.9 days	
S-P	33	1	(3.0)	4	(12.1)	28	(84.4)
						range = 1-23 days average = 6.2 days	

The ultimate answer to this vital question of development of resistance can only come with time and experience. Since October 1971, the National Malaria Eradication Project of Thailand has adopted the use of sulphormethoxine-pyrimethamine as the standard treatment for falciparum malaria. Careful monitoring of the cure rates every 6 months in areas where baseline data on cure rate are known will undoubtedly provide the most reliable early indication of emergence of resistant strains.

In areas of the world where chloroquine-resistant falciparum malaria is a significant problem, long-acting sulfonamide combinations have a definite place as an alternative treatment against these strains. From the experience in Thailand, it is our view that the rational field use of such combinations should be guided by the following considerations:

1. The recurrence of patent infection with asexual forms of falciparum malaria within 1 month following treatment with a presumptive dose of chloroquine (10 mg/kg base) is sufficient indication, in the context of an operational malaria eradication program, of chloroquine failure, suggesting the institution of long-acting sulfonamide combinations as the next treatment.

2. Until more information is available regarding the possible sporontocidal property of long-acting sulfonamide combinations, the use of primaquine as a gametocytocide should not be discarded.

3. Prior to contemplated operational use, an evaluation should be undertaken to determine susceptibility of local strains to the long-acting sulfonamide combinations.

4. Following failures to cure with such combinations, treatment with quinine with or without other antimalarial agents should be instituted

In view of the lack of demonstrated differences in antimalarial property between the two combinations evaluated, selection of one of the two for intended operational use by a malaria program must then be based on other factors, the most important being the relative cost.

It is apparent that sulfonamide combinations, because of their known limitations, are not the final solution to the problem of drug-resistant falciparum malaria. Until a better treatment is developed, such combinations can, if used judiciously, fill exigent needs

ACKNOWLEDGMENTS

We thank Dr. William E. Collins for performing the indirect fluorescent antibody determinations. We also wish to thank the staff of the National Malaria Eradication Project of Thailand for their cooperation and valuable assistance in the conduct of the study.

REFERENCES

1. Harinasuta, T., Migasen, S., and Boonnag, D., 1962. Chloroquine resistance in *Plasmodium falciparum* in Thailand. *Proc. 1st Regional Symposium on Scientific Knowledge of Tropical Parasites, Singapore* UNESCO, 148.
2. Harinasuta, T., Viravan, C., and Reid, H. A., 1967. Sulphormethoxine in chloroquine-resistant falciparum malaria in Thailand. *Lancet*, 1: 117-119.
3. Bourke, A. T. C., Puhomchareon, S., Cadigan, F. C., Gould, D. J., and Pinswasdi, K., 1966. Prevalence of malaria exhibiting reduced sensitivity to chloroquine in Southern Thailand. *Trans. R. Soc. Trop. Med. Hyg.*, 60: 225-230.
4. Chin, W., Thailand Malaria Operational Research Unit Annual Report for FY-70.
5. Chin, W., Thailand Malaria Operational Research Unit Annual Report for FY-69.
6. Hickman, R. L., Colwell, E. J., Phintuyothin, P., Sadudee, S., and Kosakal, S., Annual Progress Report for 1970-71, SEATO Medical Research Laboratory, pp. 203-208.
7. Colwell, E. J., Hickman, R. L., and Kosakal, S., 1972. Tetracycline treatment of chloroquine-resistant falciparum malaria in Thailand. *J. Am. Med. Assoc.*, 220: 684-686.
8. Hurly, M. G. D., 1959. Potentiation of pyrimethamine by sulfadiazine in human malaria. *Trans. R. Soc. Trop. Med. Hyg.*, 53: 412-413.
9. Chin, W., Contacos, P. G., Coatney, G. R., and King, H. K., 1966. The evaluation of sulfonamides, alone or in combination with pyrimethamine, in the treatment of multi-resistant falciparum malaria. *Am. J. Trop. Med. Hyg.*, 15: 823-829.
10. Clyde, D. F., Miller, R. M., Schwartz, A. R., and Levine, M. M., 1971. Treatment of falciparum malaria with sulfalene and trimethoprim. *Am. J. Trop. Med. Hyg.*, 20: 804-810.
11. Richards, W. H. G., 1968. Resistance of *P. berghei* to pyrimethamine plus sulphonamides. Summary of contribution to malaria seminar, May 30, 1968, London School of Hygiene and Tropical Medicine.
12. Verdrager, J., Riche, A., and Chheang, C. M., 1968. Action de l'association diaphenylsulfone-pyriméthamine sur le paludisme à *Plasmodium falciparum* au Cambodge. *Bull. WHO.* 40: 319-324.
13. Chin, W., Contacos, P. G., and Coatney, G. R., 1970. Malaria therapy. *Trans. R. Soc. Trop. Med. Hyg.*, 64: 461-462.